BRIEFER ARTICLES

NOTES ON EFFECT OF DYES ON *ENDOTHIA PARASITICA*

Some experiments were made in growing the chestnut blight fungus, *Endothia parasitica*, in a liquid medium to which stains had been added. The dyes were congo red, trypan blue, methylene blue, and neutral red plus 7 per cent NaCl, all "vital stains." They were added to a nutrient medium, Pasteur's solution. This solution was not a particularly good one for the cultivation of the fungus. Congo red, trypan blue, and methylene blue were used in 1/1000 of 1 per cent solutions. The dilution of neutral red plus 7 per cent NaCl was not known. This solution had been successfully used in the vital staining of some animals of the lower orders, and it was tried on the fungus by diluting it 1 cc. to 500 cc. of Pasteur's solution. The cultures were started from conidia mixed with a little mycelium taken from a test tube culture.

Record of experiments

CONGO RED (1/1000 of 1 per cent congo red[1] in Pasteur's solution).—Conidia germinated and produced normal mycelium. The hyphae became red colored. When the cultures were 5 days old, hyphae and medium had the same color. Reaction of medium acid to litmus paper. Eleven days after inoculation the medium had turned a pale yellow color, almost colorless, and clear. The medium was acid to litmus paper. The red colored mycelium which had been spreading steadily over the surface of the medium showed in sharp contrast. Seven days later the fully and normally developed fungus had produced pycnidia and conidia. The mycelium in the cultures appeared a pinkish red, and the pycnidia looked yellow. Under the microscope, the color of the hyphae was red to opaque. The colored hyphae turned blue at once on being placed in $\frac{1}{3}$ per cent sulphuric acid and later lost their color. The pycnidia on being tested in sulphuric acid varied in reaction; some of them turned blue, some did not. The conidia in the crushed fruits showed no color.

TRYPAN BLUE (1/1000 of 1 per cent trypan[2] blue in Pasteur's solution).—All the conidia stained a deep blue, so that their growth could

[1] G. Grübler and Company.

[2] Meister Lucius and Brüning Hoechst.

be followed readily. They swelled, germinated, and produced normal mycelia. The hyphae were a deep blue; the growing tips were opaque to pale blue. Their blue color deepened with age. About 12 days after inoculation the medium lost its blue color, turning light yellow; the blue hyphae with uncolored pycnidia floated on its surface.

METHYLENE BLUE (1/1000 of 1 per cent methylene blue[3] in Pasteur's solution).—The majority of conidia did not germinate, but instead developed vacuoles. The hyphae produced did not stain, except when the cells were dead. The plasmolyzed contents of the dead cells gathered in a blue clump in the center. These isolated dead cells distributed throughout the mycelium gave it a pale blue color. While the cultures grew more vigorously than the controls, they did not grow as well as the congo red and trypan blue cultures. No fruits were produced.

NEUTRAL RED (neutral red plus 7 per cent NaCl solution diluted 1 to 500 parts Pasteur's solution).—A few conidia germinated and grew. The cultures were as good as the controls. No fruits. Hyphae uncolored.

CONTROL (Pasteur's solution).—Conidia germinated, produced mycelium, but no pycnidia.

In the case of the congo red and trypan blue cultures, it was thought that the mycelium had gradually stored up all the dye in the medium. Neutral red and salt did not stain. In those cases in which methylene blue penetrated the cells, it apparently was fatal. The fact that the solutions containing the stains supported the fungus better than the control medium seems to indicate one of two things: either the toxicity of the dyes in the concentrations used (with the exception of the neutral red and salt) was enough to be a stimulus, or the dyes may have counteracted the elements in Pasteur's solution inhibitive to the growth of the fungus and so allowed the mycelium a better development.

PFEFFER,[4] in a series of experiments with methylene blue, found the dye accumulated in the cell sap rather than in the protoplast of the cell. The substances which render the storage possible, he says, are not always identical; the two which are best known are tannin and phloroglucin. Mycelium taken from cornmeal agar cultures was tested for the presence of tannin and phloroglucin. There was no evidence of these two substances in the vegetative cells. The reagents used were ferric sulphate, hydrogen peroxide and ferrous sulphate, copper acetate, ferrous sulphate, and boiling potassium bichromate.

[3] G. Grübler and Company.

[4] PFEFFER, W., Untersuchungen Botanischen Institut zu Tübingen. 1886. II.

Mycelia growing in a congo red 1 to 200 parts maltose solution were examined to see whether the dye had accumulated in the vacuoles or protoplast of the cell. The mycelium had not behaved with methylene blue as with the two colloids, but this gave no indication as to the deposition of the pigments.

The fungus grew in the solution, but not vigorously. The mycelium was so deeply stained as to be reddish black. Under the microscope the conidia and older cells of the hyphae were dark red, while the youngest cells were a pale pink. Treatment with 50 per cent nitric acid showed by its blue colored reaction that most of the pigment was in the walls of all the cells, only less in the younger cells. The hyphae were plasmolyzed with a NaCl solution and also by drying; the contracted protoplasm in the center of the cells was red, the cell wall looking white in contrast. This first was noticed in the younger cells, the quantity of pigment in the older cell walls having obscured the color of the protoplast, until the last stages of plasmolysis had been reached. Sulphuric acid, nitric, or hydrochloric produced besides a blue color, what was thought might be a blue precipitate. These very small spots, seen with the oil immersion lens, were on the cell walls and inside on the plasmolyzed protoplast. Glycerin caused the color to stream from the mycelium. Sodium hydroxide, while it brightened the red, also caused the color to diffuse into the surrounding solution. Throughout all these reactions glistening white granules in the protoplasts could be seen.

It would seem from these reactions that a great deal of the congo red accumulated in the cell walls, some passed inside the cell walls, where it appeared as though the protoplast had stored the dye in the form of minute granules. In the nutrient solutions containing congo red, the difference in the ability of fungi to store stain was so marked that contaminations could be seen at once. For instance, *Penicillium* sp., yeast, and a rod-shaped bacterium found growing in them remained unstained until dead.

It is suggested that *Endothia parasitica* (Mur.) A. and A. may be a good subject for the study of mitochondria in fungous cells.—CAROLINE RUMBOLD, *Botanical Laboratory, University of Pennsylvania.*